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BEHAVIOUR OF MALE COENAGRIONID DAMSELFLIES TOWARDS CONSPECIFIC FEMALES AT THE WATER'S EDGE (ZYGOPTERA: COENAGRIONIDAE)

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The behaviour of ♂ *Coenagrion puella* and *Xanthocnemis zealandica* towards conspecific andromorph and gynomorph ♀♀ was studied at breeding ponds in the U.K. and in New Zealand respectively. As expected, ♂ attention directed towards copulation wheels (*C. puella*) did not depend on whether the wheel contained an andromorph or a gynomorph. Similarly, ♂ attention directed towards tandem pairs (*C. puella* and *X. zealandica*) did not depend on whether the tandem contained an andromorph or a gynomorph. When individual andromorph and gynomorph ♀♀ (*C. puella* and *X. zealandica*) were released at the water's edge they experienced similar levels of attention from ♂♂. By contrast, ♂♂ (*X. zealandica*) formed significantly more tandems with gynomorphs tethered at the water's edge than with tethered andromorphs. The observations suggest that ♂♂ readily identify and intercept conspecific ♀♀ at the water's edge, particularly when in motion, and that andromorphs and gynomorphs are equally susceptible to ♂ attention. Behaviour of ♂♂ towards tethered ♀♀ may be atypical compared to that recorded under more natural conditions.

INTRODUCTION

Female-limited polymorphism, where one female morph (the andromorph) appears male-like and one or more other morphs (gynomorphs) do not, is a relatively common phenomenon amongst species of coenagrionid damselflies (CORDERO & ANDRÉS, 1996). It has been suggested that andromorphs may also mimic male behaviour (ROBERTSON, 1985; CORDERO, 1989; VAN GOSSUM et al., 2001). Thus andromorphs may gain some kind of frequency dependent selective advantage over gynomorphs if, through their mimicry, they avoid costs associated with excessive male sexual harassment.

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A number of studies have shown more male attention directed towards gynomorphs than andromorphs. ROBERTSON (1985) observed interaction between marked male and female *Ischnura ramburi* at a Florida pond, finding that gynomorphs were involved in copulations twice as often as andromorphs. He also recorded the response of males to live pinned females. In this case, gynomorphs were subject to more clasping (tandem attempts) than andromorphs but not to more grasping or circling. FINCKE (1994), observing tethered *Enallagma hageni*, found that males responded in a sexual manner (grabbed females, formed tandems, or attempted take-over of a female already in tandem) more frequently to gynomorphs than they did to andromorphs (males also responded sexually to andromorphs more frequently than they did to control males). MILLER & FINCKE (1999) recorded the reaction of male *E. ebrium* and *E. civile* to tethered conspecific females in the field. For both species, males reacted sexually more often to gynomorphs than to andromorphs. In contrast, *E. civile* males caged with conspecific andromorphs subsequently initiated significantly more sexual interaction with andromorphs (again, tests used tethered females). MILLER & FINCKE therefore proposed that prior experience is an important factor in male recognition of different female morphs. FORBES et al. (1997) presented focal male *Nehalennia irene* with tethered females and found that the males tended to grasp gynomorphs more than andromorphs. However, these authors suggest that males are more attracted to gynomorphs when female models are stationary because, in cage trials (where a single female was placed inside a small cage with 12 males) and in field trials (where encounters between damselflies were staged by herding males and females together), the same study found that males were no more likely to grasp free-moving gynomorphs than to grasp free-moving andromorphs.

Differences in the results of behavioural studies may reflect the use of differing experimental methodology (CORDERO & ANDRÉS, 2001). Clearly, under most circumstances, the use of conditions as close to natural as possible is preferable. On the other hand, andromorphs differ between species both in the accuracy of their male mimicry and in their frequency of occurrence within populations. Differences between study results may therefore also reflect true differences between species and/or populations. Here, we examined two contrasting species of coenagrionid damselfly, *Coenagrion puella* Linnaeus (Coenagrionidae: Coenagrioninae) and *Xanthocnemis zealandica* McLachlan (Coenagrionidae: Pseudagrioninae), for evidence that males harass gynomorphs more frequently than andromorphs at the water's edge. Our principal observations were conducted on unmarked, free-roaming males and females. We contrast these observations with the behaviour of free-roaming males towards tethered females.

METHODS

C. puella, the azure damselfly, is common and widely distributed in Europe south of 55°N (ASKEW, 1988). It is normally found in association with small bodies of standing water, flying from the end of April through to mid-September, with peak numbers in June and July. *X. zealandica*, the common redcoat damselfly, is endemic to New Zealand where it occurs in association with most aquatic habitats (ROWE, 1987). Flight period is from August until March, peaking from November onwards. Females of both species are polymorphic, with a single andromorph and a single gynomorph being recognised.

All observations were conducted on warm, calm days between 10:00 and 16:00. We defined a number of categories of behaviour. An "approach" was when one animal made an investigative flight, or turn, towards another animal. A "hassle" was when an approaching animal made physical contact with the approached animal, usually by grappling the area around the thorax. A "pass" was recorded when another damselfly flew within approximately 30 cm of the focal animal but did not apparently notice, or alter the course of its flight towards, the focal animal.

Coenagrion puella – was examined at Ness Botanic Gardens, Wirral, U.K. (3°3'W, 53°16'N). The study area was a series of shallow ornamental ponds where *Ishnura elegans* also occurred. Andromorph frequency was 34% (36/104, estimated by counting all animals seen along a transect through the gardens walked on 16 days).

Observation of copulating animals and of ovipositing tandem pairs was conducted at the ponds. Copulations were observed for periods of up to 10 minutes on 4, 5, 9, 12, 16, 26th July and 1st August 2001. The number of "approaches" and the number of "hassles" by single males towards the focal female were recorded minute by minute. The copulating pair was then caught to determine female morphotype. We attempted to watch a copulation including an andromorph and then a copulation including a gynomorph or vice versa. However, this was not always possible because of difficulty determining female morphotype whilst animals were in the copulation wheel position. Seven andromorph and 16 gynomorph copulations were watched for a total of 65 minutes and 150 minutes respectively.

Tandem pairs were followed for periods of up to 10 minutes (on 27, 28th June and 2, 3, 4, 9, 12th July 2001), during which time the number of "approaches" and the number of "hassles" from single males were recorded minute by minute. In most cases observation of a tandem including a female of one morphotype followed observation of a tandem including a female of the other morphotype. In total we watched 16 andromorph tandems (for 150 minutes overall) and 16 gynomorph tandems (for 150 minutes overall).

To look at the behaviour of males towards single females at the water's edge we caught 58 females (18 andromorphs and 40 gynomorphs) at one pond and released them at another, approximately 25m distant. The point of release was always the same. Females were caught in tandem, separated, placed in a holding cage (20 x 40 x 50cm wooden frame covered with dark coloured mosquito netting; the maximum number of animals in the cage at any one time was 12; the maximum time spent in the cage by each animal was 20 minutes), transported and then sequentially released (where possible release of an andromorph was followed by release of a gynomorph or vice versa). Two observers were involved. One observer placed an individual female onto pond-side vegetation. As soon as the female was released, the other observer recorded events second-by-second until either a male formed a tandem with the focal animal, or the focal animal was lost from sight. Twenty males were also released at the pond. They were caught and treated in the same way as the females, except that the observation period was terminated either after 5 minutes or if the male was lost from sight. Releases were carried out on 3, 6, 16, 23, 26th July 2001.

Xanthocnemis zealandica – was examined on the northern outskirts of Christchurch, New Zealand. The site was a shallow lake surrounded by maintained lawn and trees at The Groynes recreation area (172°36'E, 43°27'S). *X. zealandica* was the predominant damselfly but *Austrolestes colensonis* White was also present. Andromorph frequency was 10% (55/554, estimated by counting all animals seen around the edge of the lake on three separate days).

Tandem pairs were observed between 10:30 and 14:40 on 3 days (27, 29, 30th November 2001). Two observers were involved. All pairs were followed for 10-minute periods and the number of "approaches" and the number of "hassles" from single males recorded (minute by minute). Observation of a tandem including a female of one morphotype was always followed by observation of a tandem including a female of the other morphotype. In total, we watched 15 andromorph tandems (150 minutes overall) and 15 gynomorph tandems (150 minutes overall).

The behaviour of single males towards single females at the water's edge was examined in two ways: by watching tethered animals and by watching releases. Three observers were involved. For observation of tethered animals an andromorph, a gynomorph and a male were caught and stuck by the legs to the end of each of 3 sticks (the sticks were 8 mm x 0.9 m green plastic covered garden canes, the adhesive was a small strip of double-sided sticky tape). Once the animals were secured in a perching position, any remaining exposed areas of adhesive were covered over with blades of grass. To start an observational run, the 3 sticks were positioned 4 to 5 m apart along a suitable length of bank so that the focal animal was aligned horizontally 20 to 30 cm above the water, at right angles to the bank. Each observer then recorded, second-by-second, all activity centred on his focal animal for a synchronised 20-minute period (males that formed tandems with focal animals were removed only at the end of the 20-minute period). The position of the sticks was then changed between observers and the process repeated until each observer had watched each focal animal. This constituted one observational run. At the end of each run the animals were released. For a new run, new animals were caught and a different position along the bank was used. In total, we carried out 10 observational runs (i.e. 30 separate 20 minute observations of individuals on sticks) on 16, 18th November and 12, 13, 14, 15th December 2001.

For releases, 50 andromorphs and 50 gynomorphs were caught in tandem. They were then separated and immediately prepared for release. The release platform was the end of a stick at the waters edge (the stick was a garden cane, as used and positioned before). Events were recorded second-by-second until either a male formed a tandem with the focal animal, or the focal animal was lost from sight. No holding cage was involved and the position of release was varied around the water's edge. The release of an andromorph was always followed by the release of a gynomorph, or vice versa. We also released 10 males (in this case observation was terminated either after 5 minutes or if the male was lost from sight). Releases were carried out on 15, 16th December 2001.

RESULTS

COPULATIONS

Copulating gynomorph and andromorph *C. puella* experienced similar levels of attention from other conspecific males (Fig. 1). We did not observe any heterospecific interactions. All copulations took place in prominent sun-exposed positions, at or within 2-3 m of the waters edge where single male densities tended to be highest. There was no significant difference between the mean number of approaches towards andromorph and gynomorph copulations ($t_{21} = 0.4, p = 0.703$). There was no significant difference between the mean number of hassles directed towards andromorph and gynomorph copulations ($t_{21} = 0.6, p = 0.574$).

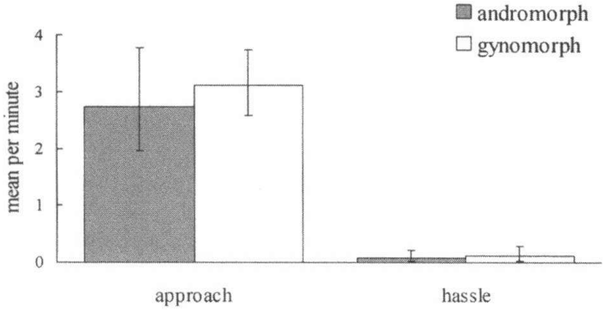

Fig. 1. Mean number of approaches and hassles per minute by single conspecific males towards copulating andromorph and gynomorph *C. puella*. – [Error bars are ±1 standard error].

TANDEMS

For both *C. puella* and *X. zealandica*, gynomorphs and andromorphs in tandem experienced similar levels of attention from other conspecific males (Fig. 2). Females in tandem spent the majority of time ovipositing, but also rested for short periods on emergent

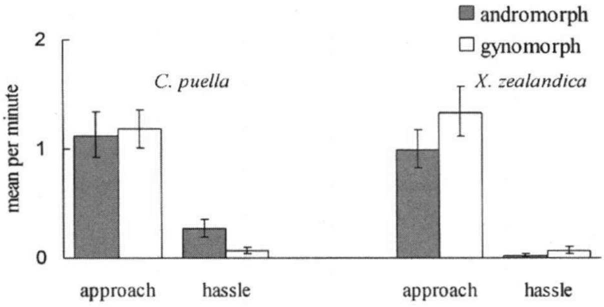

Fig. 2. Mean number of approaches and hassles per minute by single conspecific males towards andromorphs and gynomorphs in tandem (*C. puella* and *X. zealandica*). – [Error bars are ±1 standard error].

macrophytes and waterside vegetation. For both species there was no significant difference between the mean number of approaches towards andromorph and gynomorph tandems (*C. puella* $t_{30} = 0.5$, $p = 0.638$; *X. zealandica* $t_{28} = 1.0$, $p = 0.334$), or the mean number of hassles (*C. puella* $t_{30} = 2.0$, $p = 0.053$; *X. zealandica* $t_{28} = 1.4$, $p = 0.175$). We did not observe any interaction between male *I. elegans* and focal *C. puella* females in tandem. Male *A. colensonis* were twice seen to approach gynomorph *X. zealandica* tandems but no hassle was involved.

SINGLE FEMALES AT THE WATER'S EDGE

The behaviour of males towards single females at the waters edge depended on how the females were presented. Males formed significantly more tandems with gynomorphs (*X. zealandica*) tethered at the water's edge than with andromorphs (see below). In contrast, when females were released (*C. puella* and *X. zealandica*) and allowed to move freely, males formed tandems with both female morphotypes at similar rates (see below). We did not observe any interaction between focal females and heterospecific males during these parts of the study.

Table I provides a summary of events recorded during observation of tethered *X. zealandica*. The number of passes to andromorphs, gynomorphs and males and the number of approaches to andromorphs, gynomorphs and males by other conspecific males did not differ significantly ($\chi^2 = 3.5$, $df = 2$, $p = 0.171$; $\chi^2 = 3.0$, $df = 2$, $p = 0.218$; respectively). Tethered males experienced relatively little hassle from other males, although in one instance a tandem attempt towards a tethered male was recorded. Hassle rate towards the two female morphotypes was similar ($\chi^2 = 1.7$, $df = 1$, $p = 0.198$). However,

Table I

Events recorded whilst observing tethered *X. zealandica*

Model	Passes by conspecific males	By approaches conspecific males	By hassles conspecific males	By tandems formed
Andromorph	175	83	31	12
Gynomorph	194	94	42	26
Male	212	107	10	1

Table II

Events recorded whilst observing *C. puella* and *X. zealandica* female releases

Species	Form	Immediate flight away from water	Flight away from release point; stayed at water; no incident	Tandem whilst resting	Tandem whilst flying
C. puella	Andromorph	10	0	5	2
	Gynomorph	25	0	18	4
X. zealandica	Andromorph	18	11	4	17
	Gynomorph	24	13	1	12

tandem formation was not: gynomorphs were involved in significantly more tandems than andromorphs ($\chi^2 = 5.2$, $df = 1$, $p = 0.023$).

On release, many females immediately flew directly away from the water (Tab. II). This behaviour was independent of female morphotype (Fisher exact tests: *C. puella*, $p = 0.780$; *X. zealandica*, $p = 0.311$). Of the 10 andromorph and 25 gynomorph *C. puella* females that immediately left the water, 2 and 5 individuals respectively were seen and chased by males. Of the 18 andromorph and 24 gynomorph *X. zealandica* females that immediately left the water, 2 and 3 individuals respectively were seen and chased by males. We did not observe the outcome of these aerial chases.

Twenty-nine *C. puella* females (7 andromorphs and 22 gynomorphs) did not immediately fly away from the water (Tab. II). Males discovered them all and tandems were formed in all cases. The mean time until interception and tandem formation was 39 seconds for andromorphs ($s = 35$, $n = 7$) and 84 seconds for gynomorphs ($s = 122$, $n = 22$). These means are not significantly different ($t_{27} = 0.8$, $p = 0.451$).

Fifty-eight *X. zealandica* females (32 andromorphs and 26 gynomorphs) did not immediately fly away from the water (Tab. II). Of these, 11 andromorphs and 13 gynomorphs flew away from the point of release onto other waterside vegetation but were not noticed by males. All remaining animals received sexual attention from males and tandems were formed in all cases. The number of andromorph tandems formed and the number of gynomorph tandems formed was similar ($\chi^2 = 1.9$, df = 1, $p = 0.170$) and the mean time (seconds) until interception did not significantly differ between female morphotypes (andromorph mean = 50, $s = 75$, $n = 21$; gynomorph mean = 88, $s = 122$, $n = 13$; $t_{32} = 1.1$, $p = 0.289$).

We recorded no instances of hassle from resident males (conspecific and heterospecific) towards males released at the water's edge. Of the 20 *C. puella* individuals released 2 immediately flew away from the water; the others remained and took up the usual patrolling activity without confrontation. Of the 10 *X. zealandica* males released, 2 immediately flew away and were lost from sight. The remaining 8 individuals moved to nearby waterside vegetation, again without confrontation by resident males.

DISCUSSION

Male *C. puella* and *X. zealandica* did not significantly differ in their behaviour towards free-roaming conspecific andromorphs and gynomorphs. They appeared to rapidly recognise females of both morphotypes as potential sexual partners. This recognition was often achieved at some distance (2-3m) and appeared independent of whether the identified female was perching or flying. Once spotted, females were intercepted directly: males did not need to make noticeable initial investigative approach flights towards females to be sure of their identification. Females of both species are larger than males but andromorphs and gynomorphs do not differ in mean size. Furthermore, compared with males, females are characteristically more ponderous in flight and general behaviour. A major signal of a female's receptivity to sexual approach is probably simply her arrival at the water's edge. The likelihood of a released female immediately fleeing from the water's edge was independent of her morphotype.

The rate of male activity directed towards copulating gynomorphs was similar to that directed towards copulating andromorphs. This is not surprising if males are aware that copulation wheels should almost always include a conspecific female but, to our knowledge, this is the first time this hypothesis has been tested. Copulation wheels were sometimes disturbed into flight by the attention of other males. Occasionally the wheel broke to a tandem but copulation was normally quickly resumed. Andromorph and gynomorph tandems also received similar rates of attention from other males. Again, males are probably aware that under almost all circumstances a tandem pair must include a conspecific female. Whilst there is little question that males were trying to get at females in a tandem, this was not a fruitful tactic. Males were never observed to break tandems or copulating pairs and steal the female. The persistence of males in their harassment of tandems and copulations therefore seemed rather futile, although FINCKE (1982) notes that such agonistic behaviour may sometimes be successful because she observed the displacement of male *E. hageni* in tandem with tethered females. We observed no obvious physical damage to tandem or copulation wheel members due to the actions of single roving males.

Andromorphs of some coenagrionid species (particularly ischnurans) can be exceptionally good male colour mimics. It is often difficult to distinguish such andromorphs without close scrutiny. On a relative scale, andromorphs of *C. puella* and *X. zealandica* are less good colour mimics. Moreover, both *C. puella* and *X. zealandica* were predominant at the study sites and the later differed greatly in body-size and behaviour to sympatric *A. colensonis*. These factors may have made it relatively easy for our males to quickly recognise both andromorphs and gynomorphs as females. In species where andromorphs are exceptional male mimics and/or in communities containing equitable densities of similar damselfly species, it may be the case that males have greater difficulty in identifying females and that andromorphs gain some degree of protection from male harassment because of their appearance and/or behaviour.

During experiments with tethered *X. zealandica*, conspecific males formed tandems with gynomorphs significantly more often than with andromorphs. This result supports those of other studies using similarly presented animals (e.g. ROBERTSON, 1985; FINCKE, 1994; FORBES et al., 1997; CORDERO, 1989; CORDERO et al., 1998;

GORB, 1998; MILLER & FINCKE, 1999), and raises two questions: (1) why did it occur and (2) why is it at odds with the outcome of our releases of females? One possibility, which would require further testing, is that behavioural as well as morphological cues aid in the identification of sexual partners. Thus when they are stationary, andromorphs may gain greater protection from harassment compared to gynomorphs but behavioural cues more strongly reveal their identity when they are flying. Whatever the underlying causes of the discrepancy it highlights the need for more work to elucidate the ecological significance of the various estimates of preference in this system (see CORDERO & ANDRÉS, 2001). Our own impression is that measures of preference that allow females to freely roam in their natural habitats (as opposed to using dead pinned animals or encounters in cages, say) likely provide the most meaningful indications of male response to the different female morphs.

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